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(54) Title: FXR NR1H4 NUCLEAR RECEPTOR BINDING COMPOUNDS

(57) Abstract: The present invention relates to compounds according to the general formula (I) which bind to the nuclear receptor, NR1H4, and act as agonists, antagonists or mixed agonists / antagonists of the NR1H4 receptor. The invention further relates to the treatment of diseases and/or conditions through binding of the nuclear receptor by the compounds.

FXR NR1H4 NUCLEAR RECEPTOR BINDING COMPOUNDS

Field of the Invention

[001] The present invention relates to compounds according to the general formulae (1), (2), (3) and (4) which bind to the NR1H4 receptor and act as agonists, antagonists or mixed agonists / antagonists of the NR1H4 receptor. The invention further relates to the treatment of diseases and/or conditions through binding of said nuclear receptor by said compounds and the production of medicaments using said compounds.

Background of the Invention

[002] Multicellular organisms are dependent on advanced mechanisms of information transfer between cells and body compartments. The information that is transmitted can be highly complex and can result in the alteration of genetic programs involved in cellular differentiation, proliferation, or reproduction. The signals, or hormones, are often simple molecules, such as peptides, fatty acid, or cholesterol derivatives.

[003] Many of these signals produce their effects by ultimately changing the transcription of specific genes. One well-studied group of proteins that mediate a cells response to a variety of signals is the family of transcription factors known as nuclear receptors, hereinafter referred to often as "NR". Members of this group include receptors for steroid hormones, vitamin D, ecdysone, cis and trans retinoic acid, thyroid hormone, bile acids, cholesterol-derivatives, fatty acids (and other peroxisomal proliferators), as well as so-called orphan receptors, proteins that are structurally similar to other members of this group, but for which no ligands are known (Escriva, H. et al., Ligand binding was acquired during evolution of nuclear receptors, PNAS, 94, 6803 – 6808, 1997). Orphan receptors may be indicative of unknown signaling pathways in the cell or may be nuclear receptors that function without ligand activation. The activation of transcription by some of these orphan receptors may occur in the

absence of an exogenous ligand and/or through signal transduction pathways originating from the cell surface (Mangelsdorf, D. J. et al., *The nuclear receptor superfamily: the second decade*, Cell 83, 835-839, 1995).

[004] In general, three functional domains have been defined in NRs. An amino terminal domain is believed to have some regulatory function. A DNA-binding domain hereinafter referred to as "DBD" usually comprises two zinc finger elements and recognizes a specific Hormone Responsive Element hereinafter referred to as "HRE" within the promoters of responsive genes. Specific amino acid residues in the "DBD" have been shown to confer DNA sequence binding specificity (Schena, M. & Yamamoto, K.R., Mammalian Glucocorticoid Receptor Derivatives Enhance Transcription in Yeast, Science, 241:965-967, 1988). A Ligand-binding-domain hereinafter referred to as "LBD" is at the carboxy-terminal region of known NRs. In the absence of hormone, the LBD of some but not all NRs appears to interfere with the interaction of the DBD with its HRE. Hormone binding seems to result in a conformational change in the NR and thus opens this interference (Brzozowski et al., *Molecular basis of agonism and antagonism in the oestrogen receptor*, Nature, 389, 753 – 758, 1997; Wagner et al., *A structural role for hormone in the thyroid hormone receptor*, Nature, 378, 690 – 697, 1995). A NR without the HBD constitutively activates transcription but at a low level.

[005] Coactivators or transcriptional activators are proposed to bridge between sequence specific transcription factors and the basal transcription machinery and in addition to influence the chromatin structure of a target cell. Several proteins like SRC-1, ACTR, and Grip1 interact with NRs in a ligand enhanced manner (Heery et al., *A signature motif in transcriptional coactivators mediates binding to nuclear receptors*, Nature, 387, 733 – 736; Heinzel et al., *A complex containing N-CoR, mSin3 and histone deacetylase mediates transcriptional repression*, Nature 387, 43 – 47, 1997). Furthermore, the physical interaction with repressing receptor-interacting proteins or corepressors has been demonstrated (Xu et al., Coactivator and *Corepressor complexes in nuclear receptor function*, Curr Opin Genet Dev, 9 (2), 140 – 147, 1999).

[006] Nuclear receptor modulators like steroid hormones affect the growth and function of specific cells by binding to intracellular receptors and forming nuclear receptor-ligand

complexes. Nuclear receptor-hormone complexes then interact with a hormone response element (HRE) in the control region of specific genes and alter specific gene expression.

[007] The Farnesoid X Receptor alpha (FXR; hereinafter also often referred to as NR1H4 when referring to the human receptor) is a prototypical type 2 nuclear receptor which activates genes upon binding to promoter region of target genes in a heterodimeric fashion with Retinoid X Receptor (hereinafter RXR, Forman et al., Cell, 81, 687-93, 1995). The relevant physiological ligands of NR1H4 seem to be bile acids (Makishima et al., Science, 284, 1362-65, 1999; Parks et al., Science, 284, 1365-68, 1999). The most potent is chenodeoxycholic acid, which regulates the expression of several genes that participate in bile acid homeostasis.

[008] Farnesol, originally described to activate the rat ortholog at high concentration does not activate the human or mouse receptor. FXR is expressed in the liver, small intestine, colon, ovary, adrenal gland and kidney. Like LXR-α, NR1H4 is involved in autocrine signaling.

[009] FXR is proposed to be a nuclear bile acid sensor. As a result, it modulates both, the synthetic output of bile acids from the liver and their recycling in the intestine (by regulating bile acid binding protein). Upon activation (e.g. binding of chenodeoxycholic acid) it influences the conversion of dietary cholesterol into bile acids by by inhibiting the transcription of key genes which are involved in bile acid synthesis such as CYP7A1 or in bile acid transport across the hepatocyte membranes such as the bile acid transporters BSEP (=Bile Salt Export Pump) and NTCP (Na-Taurocholate Co-Transporter). This seems to be a major mechanism of feedback regulation onto bile acid synthesis. Moreover, NR1H4 seems to be the crucial receptor for maintaining bile acid homeostasis within the hepatocyte and therefore might be an appropriate drug target to treat diseases that result from impaired bile acid production, impaired export into the bile canaliculi or impaired bile flow in general such as cholestatic conditions. Loss of function of NR1H4 results in major changes in bile acid homeostasis on the organism level (Lu, et al., Mol Cell. (2000) 6(3):507-15; Goodwin, et al., Mol Cell. (2000) 6(3):517-26; Sinal, et al., Cell (2000) 15;102(6):731-44).

[010] The synthetic compounds, 1,1-bisphosphonate esters, appear to display a number of similar activities to the two identified prototypes of natural FXR agonists, farnesol, and chenodeoxycholic acid. Like farnesol, the 1,1- bisphosphonate esters increase the rate of 3-

Hydroxy-3-methylglutaryl-CoA (HMG-CoA) Reductase degradation and like bile acids they induce the expression of the Intestinal Bile Acid Binding Protein (I-BABP) and repress the cholesterol 7 α-hydroxylase gene. Certain 1,1-bisphosphonate esters also bind to FXR. (Niesor et al., Curr Pharm Des,7(4):231-59, 2001)..That means that activation of FXR could lead to opposing effects (lowering the rate of cholesterol synthesis by increasing degradation of HMG-CoA Reductase and increasing the cholesterol pool by inhibition of cholesterol degradation into bile acids). The FXR agonist chenodeoxycholic acid does not change cholesterol and lipoprotein levels significantly in patients, although a repression of bile acid synthesis as well as a decreased HMG-CoA Reductase activity was observed (Einarsson et al., Hepatology, 33(5), 1189-93, 2001) confirming that cellular cholesterol synthesis and degradation are controlled by numerous regulatory loops including the coordinate regulation of HMGCoA reductase and cholesterol 7α-hydroxylase and that compounds modulating FXR acitvity might have different effects on blood lipid parameters.

[011] In the course of functional analysis of certain 1,1-bisphosphonate esters, it was shown that these compounds, which are known to bind to FXR also induce apoptosis in a variety of cell types, similar to the isporenoids farnesol and geranylgeraniol, which are also known as weak FXR binders (Flach et al., Biochem Biophys Res Com, 270, 240-46, 2000).

[012] To date only very few compounds have been described which bind the NR1H4 receptor and thus show utility for treating diseases or conditions which are due to or influenced by said nuclear receptor (Maloney at al., J Med Chem, 10; 43(16):2971-4, 2000).

[013] It is currently believed that FXR agonists might be useful to treat cholestatic conditions because they result in an upregulation of bile acid transport activity across the canalicular hepatocyte membrane (Plass, et al., Hepatology. (2002) 35(3):589-96; Willson, et al., Med Res Rev. (2001) 21(6):513-22) . In contrast, it is believed that compounds that act as FXR antagonists or at least as mixed agonists / antagonists might reduce total serum cholesterol (Urizar, et al., Science (2002) 31;296(5573):1703-6).

[014] It was thus an object of the present invention to provide for novel NR1H4 binding compounds. It was thus an object of the present invention to provide for compounds which by means of binding the NR1H4 receptor act as agonist or antagonist or mixed agonist / antagonist

of said receptor and thus show utility for treating diseases or conditions which are due to or influenced by said nuclear receptor.

[015] It was further an object of the invention to provide for compounds which may be used for the manufacture of a medicament for the treatment of cholesterol or bile acid associated conditions or diseases. In a preferred embodiment of the invention it was an object of the invention to provide for cholesterol lowering or anti-cholestatic compounds. It was also an object of the invention to provide for compounds that may be used for the manufacture of anticancer medicaments or apoptosis-inducing medicaments in general.

[016] It was further an object of the invention to provide for compounds which are orally available and can be used for an oral treatment of the diseases mentioned afore.

[017] The foregoing merely summarizes certain aspects of the present invention and is not intended, nor should it be construed, to limit the invention in any manner. All patents and other publications recited herein are hereby incorporated by reference in their entirety.

Brief Description of the Drawings

[018] Figure 1 shows the synthesis of the compounds according to the invention and as described in Example 2.

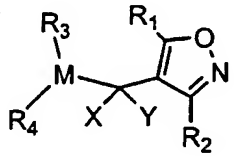
[019] Figure 2A is the amino acid sequence of the FXR protein, a portion of which was used for cloning as described in the examples (SEQ ID NO. 1). Figure 2B shows the nucleotide sequence (SEQ ID NO. 2) of FXR mRNA. Figure 2C is the amino acid sequence of TIF2 (ACC. NO: XM_011633 REFSEQ DB)(SEQ ID NO.3) and Figure 2D shows the nucleotide sequence of TIF2 mRNA (SEQ ID NO. 4).

[020] Figure 3 shows a dose-dependent transactivation (EC50 ~ 1 μM) of the reporter gene, luciferase, by FXR upon administration of different concentrations of compounds. Compounds LN169 and LN6734 that are within the scope of this patent application demonstrate a much higher potency in this assay than the published compound GW4064 or the natural ligand CDCA.

[021] Figure 4 shows a table with the underlying data to Fig 3.

Detailed Description of the Invention

[022] The invention provides for a compound including resolved diastereoisomers and enantiomers and tautomers, pharmaceutical acceptable salts or solvates thereof (hereinafter also referred to as the "compounds according to the invention"), having the following formula (I):



(I)

wherein,

[023] R_1 is hydrogen, C_1 to C_8 alkyl, C_1 to C_8 substituted alkyl, phenyl, substituted phenyl, heteroaryl, substituted heteroaryl, heterocycle, substituted heterocycle, naphthyl or substituted naphthyl;

[024] R_2 is hydrogen C_1 to C_8 alkyl, C_1 to C_8 substituted alkyl, phenyl, substituted phenyl, heteroaryl, substituted heteroaryl, heterocycle, substituted heterocycle, naphthyl or substituted naphthyl;

[025] R_3 is absent or if present selected from hydrogen, C_1 to C_8 alkyl, C_1 to C_8 substituted alkyl, C_7 to C_{12} alkylphenyl or C_7 to C_{12} substituted phenylalkyl, phenyl, C_5 to C_6 heteroaryl, C_5 to C_6 substituted heteroaryl, substituted phenyl, biphenyl, substituted biphenyl, biphenyl ether, substituted biphenyl ether, biphenyl amine, substituted biphenyl amine, naphthyl and substituted naphthyl; and

[026] R_4 is absent, or if present, selected from hydrogen, C_1 to C_8 alkyl, C_1 to C_8 substituted alkyl, C_7 to C_{12} alkylphenyl or C_7 to C_{12} substituted phenylalkyl, phenyl,

substituted phenyl, C_5 to C_6 heteroaryl, C_5 to C_6 substituted heteroaryl, biphenyl, substituted biphenyl, biphenyl ether, substituted biphenyl ether, biphenyl amine, substituted biphenyl amine, naphthyl and substituted naphthyl; M is O or N or S, however, when M is O or S, one of R_3 or R_4 must be absent; and

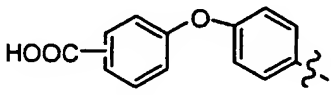
[027] X and Y are both hydrogen or both methyl or together represent a carbonyl group;

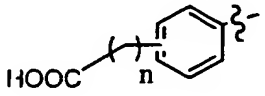
[028] R_3 and R_4 may be taken together with nitrogen to form a heterocycle or substituted heterocycle or a heteroaryl or substituted heteroaryl ring.

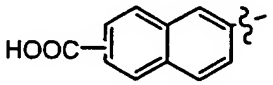
[029] In one embodiment of the present invention R_1 and R_2 in formula (I) as shown above are independently selected from the group consisting of R_1 is hydrogen, C_1 to C_8 alkyl, C_1 to C_8 substituted alkyl; R_2 is phenyl, substituted phenyl, heteroaryl, substituted heteroaryl, heterocycle, and substituted heterocycle; R_3 is absent, or if present, is select from the group consisting of hydrogen, C_1 to C_8 alkyl, C_1 to C_8 substituted alkyl, C_7 to C_{12} alkylphenyl or C_7 to C_{12} substituted phenylalkyl, phenyl, substituted phenyl, C_5 to C_6 heteroaryl, C_5 to C_6 substituted heteroaryl, naphthyl and substituted naphthyl; R_4 is absent, or if present, is select from the group consisting of hydrogen, C_1 to C_8 alkyl, C_1 to C_8 substituted alkyl, C_7 to C_{12} alkylphenyl or C_7 to C_{12} substituted phenylalkyl, phenyl, substituted phenyl, C_5 to C_6 heteroaryl, C_5 to C_6 substituted heteroaryl, naphthyl and substituted naphthyl; M is O or N or S, however, if M is O or S, one of R_3 or R_4 must be absent; X and Y are both hydrogen, or both methyl, or together represent a carbonyl group.

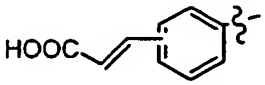
[030] In a more preferred embodiment of the present invention R_1 is hydrogen, C_1 to C_8 alkyl or C_1 to C_8 substituted alkyl, R_2 is phenyl, substituted phenyl, heteroaryl, substituted heteroaryl, heterocycle or substituted heterocycle; R_3 is absent, or if present, is hydrogen, C_1 to C_8 alkyl or C_1 to C_8 substituted alkyl; R_4 is C_7 to C_{12} substituted phenylalkyl, substituted phenyl, C_5 to C_6 substituted heteroaryl or substituted naphthyl; M is O or N or S, however, if M is O or S, one of R_3 or R_4 must be absent; X and Y are both hydrogen, both methyl, or together represent a carbonyl group.

[031] In a more preferred embodiment of the invention, R_1 is hydrogen, C_1 to C_8 alkyl, C_1 to C_8 substituted alkyl, alkyl phenyl, substituted phenyl, C_5 to C_6 heteroaryl, C_5 to C_6 substituted heteroaryl, naphthyl or substituted naphthyl; R_2 is substituted phenyl, C_5 to C_6 heteroaryl or C_5 to C_6 substituted heteroaryl; R_3 has one of the following structures;









[032] R4 is absent;

[033] M =O,

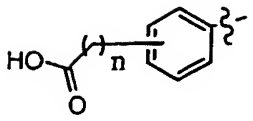
[034] both X and Y are hydrogen; and

[035] n is an integer from 0 to 8, preferably 1 to 6, and most preferably, 1 to 4.

[036] The symbol in the above formulas and in formula (II) below:

represents a fragment and covalent linkage between the fragment and the aromatic ring.

[037] An even more preferred embodiment of the invention is a compound, or pharmaceutical acceptable salts or solvates thereof, wherein R_1 is C_1 to C_8 alkyl or C_1 to C_8 substituted alkyl; R_2 is substituted phenyl; R_3 has the following formula (II):



(II)

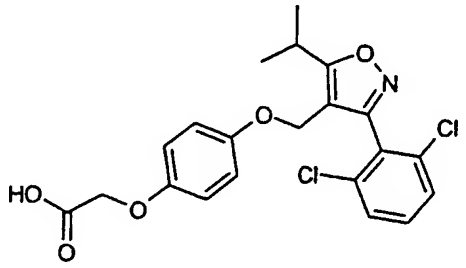
[038] R_4 is absent

[039] M = O,

[040] both X and Y are hydrogen;

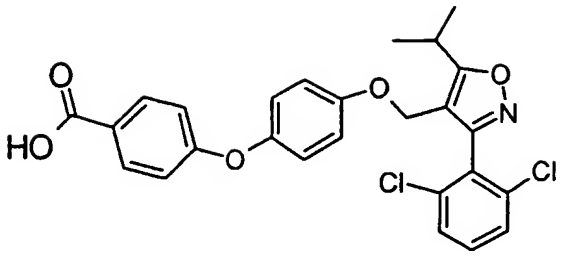
[041] and n is an integer from 0 to 8.

[042] A particularly preferred compound which may act as agonist of NR1H4 is shown in formula (II) below. The inventors have been able to demonstrate that the compound according to formula (II) has a low effective concentration at FXR with an EC_{50} of 0.23 μM wherein the EC_{50} reflects the half-maximal effective concentration, and which is higher than the EC_{50} of 0.015 μM for the published FXR agonist GW4064 (B.Goodwin et al., Molecular Cell 6, 517-526, 2000).



(II)

[043] The inventors have also found the compounds according to formulas III, IV and V below to be active as agonist of the NR1H4 human nuclear receptor (see figures for details).



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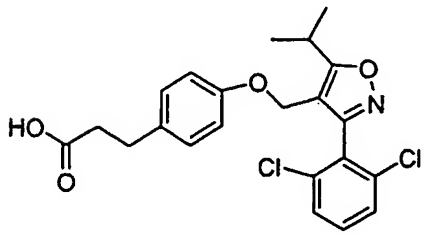

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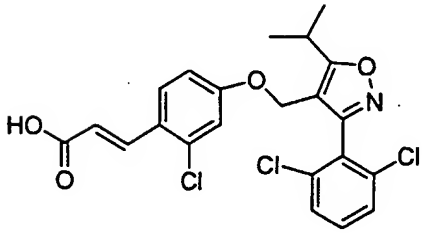


(V)

[044] The inventors have identified the compounds as well as the general structure capable of effectively binding FXR.

[045] The compounds of the invention can also exist as solvates and hydrates. Thus, these compounds may crystallize with, for example, waters of hydration, or one, a number of, or any fraction thereof of molecules of the mother liquor solvent. The solvates and hydrates of such compounds are included within the scope of this invention.

[046] The term "halogen" refers to the fluoro, chloro, bromo or iodo atoms. There can be one or more halogen, which are the same or different. Preferred halogens are chloro and fluoro.

[047] The term "C_1 to C_8 alkyl" denotes such radicals as methyl, ethyl, n-propyl, isopropyl, n-butyl, iso-butyl, sec-butyl, tert-butyl, amyl, tert-amyl, hexyl , n-heptyl, 2-heptyl, 3-heptyl, 4-heptyl, 2-methyl-1-hexyl, 2-methyl-2-hexyl, 2-methyl-3-hexyl, n-octyl and the like.

[048] The term "C_1 to C_8 substituted alkyl" denotes that the above C_1 to C_8 alkyl groups are substituted by one or more, and preferably one or two, halogen, hydroxy, protected hydroxy, oxo, protected oxo, C_3 to C_7 cycloalkyl, phenyl, naphthyl, amino, protected amino, monosubstituted amino, protected monosubstituted amino, disubstituted amino, guanidino, protected guanidino, heterocyclic ring, substituted heterocyclic ring, C_1 to C_8 alkoxy, C_1 to C_8 acyl, C_1 to C_8 acyloxy, nitro, carboxy, protected carboxy, carbamoyl, carboxamide, protected carboxamide, N-(C_1 to C_6 alkyl)carboxamide, protected N-(C_1 to C_6 alkyl)carboxamide, N,N-di(C_1 to C_6 alkyl)carboxamide, cyano, methylsulfonylamino, thiol, C_1 to C_4 alkylthio or C_1 to C_4 alkylsulfonyl groups. The substituted alkyl groups may be substituted once or more, and preferably once or twice, with the same or with different substituents.

[049] Examples of the above substituted alkyl groups include the 2-oxo-prop-1-yl, 3-oxo-but-1-yl, cyanomethyl, nitromethyl, chloromethyl, hydroxymethyl, tetrahydropyranyl-oxymethyl, trityloxymethyl, propionyloxymethyl, amino, methylamino, aminomethyl, dimethylamino, carboxymethyl, allyloxycarbonylmethyl, allyloxycarbonylaminomethyl, methoxymethyl, ethoxymethyl, t-butoxymethyl, acetoxymethyl, 4-carboxybutyl, 5-carboxypentyl, 6-carboxyhexyl, chloromethyl, bromomethyl, iodomethyl, trifluoromethyl, 6-hydroxyhexyl, 2,4-dichloro(n-butyl), 2-aminopropyl, 1-chloroethyl, 2-chloroethyl, 1- bromoethyl, 2-chloroethyl, 1-fluoroethyl, 2-fluoroethyl, 1- iodoethyl, 2-iodoethyl, 1-chloropropyl, 2-chloropropyl, 3-chloropropyl, 1-bromopropyl, 2-bromopropyl, 3-bromopropyl, 1-fluoropropyl, 2-fluoropropyl, 3-fluoropropyl, 1- iodopropyl, 2-iodopropyl, 3-iodopropyl, 2-aminoethyl, 1- aminoethyl, N-benzoyl-2-aminoethyl, N-acetyl-2-aminoethyl, N-benzoyl-1-aminoethyl, N-acetyl-1-aminoethyl and the like.

[050] The term "substituted phenyl" specifies a phenyl group substituted with one or more, and preferably one or two, moieties chosen from the groups consisting of halogen, hydroxy, protected hydroxy, cyano, nitro, C_1 to C_8 alkyl, C_1 to C_8 substituted alkyl, C_1 to C_8 alkoxy, C_1 to C_8 substituted alkoxy, substituted phenoxy, substituted phenyl amine, C_1 to C_8 acyl, C_1 to C_8 substituted acyl, C_1 to C_8 acyloxy, carboxy, protected carboxy, carboxymethyl, protected

carboxymethyl, hydroxymethyl, protected hydroxymethyl, amino, protected amino, monosubstituted amino, protected monosubstituted amino, disubstituted amino, carboxamide, protected carboxamide, N-(C_1 to C_6 alkyl)carboxamide, protected N-(C_1 to C_6 alkyl)carboxamide, N, N-di(C_1 to C_6 alkyl)carboxamide, trifluoromethyl, N-((C_1 to C_6 alkyl)sulfonyl)amino, N-(phenylsulfonyl)amino or phenyl, wherein the phenyl is substituted or unsubstituted, such that, for example, a biphenyl results.

[051] Examples of the term "substituted phenyl" include a mono- or di (halo) phenyl group such as 2, 3 or 4-chlorophenyl, 2,6-difluorophenyl, 2,3-difluorophenyl, 2,6-dichlorophenyl, 2,5-dichlorophenyl, 3,4-dichlorophenyl, 2, 3 or 4-bromophenyl, 3,4-dibromophenyl, 3-chloro-4-fluorophenyl, 2, 3 or 4-fluorophenyl and the like; a mono or di (hydroxy) phenyl group such as 2, 3 or 4-hydroxyphenyl, 2,4-dihydroxyphenyl, the protected-hydroxy derivatives thereof and the like; a nitrophenyl group such as 2, 3 or 4-nitrophenyl; a cyanophenyl group, for example, 2, 3 or 4-cyanophenyl; a mono- or di(alkyl)phenyl group such as 2, 3 or 4-methylphenyl, 2,4-dimethylphenyl, 2, 3 or 4-(iso-propyl)phenyl, 2, 3 or 4-ethylphenyl, 2, 3 or 4-(n-propyl)phenyl and the like; a mono or di(alkoxyl)phenyl group, for example, 2,6-dimethoxyphenyl, 2, 3 or 4-methoxyphenyl, 2, 3 or 4-ethoxyphenyl, 2, 3 or 4-(isopropoxy)phenyl, 2, 3 or 4-(t-butoxy)phenyl, 3-ethoxy-4-methoxyphenyl and the like; 2, 3 or 4-trifluoromethylphenyl; a mono- or dicarboxyphenyl or (protected carboxy)phenyl group such as 2, 3 or 4-carboxyphenyl or 2,4-di(protected carboxy)phenyl; a mono-or di(hydroxymethyl)phenyl or (protected hydroxymethyl)-phenyl such as 2, 3, or 4-(protected hydroxymethyl)phenyl or 3,4-di(hydroxymethyl)phenyl; a mono- or di(aminomethyl)phenyl or (protected aminomethyl)phenyl such as 2, 3 or 4-(aminomethyl)phenyl or 2,4-(protected aminomethyl)phenyl; or a mono- or di(N-(methylsulfonyl-amino))phenyl such as 2, 3 or 4-(N-(methylsulfonylamino))phenyl, 4-(4'-carboxy phenoxy)-phenyl, 4-(4'-protected carboxy phenoxy)-phenyl, 4-(3'-carboxy phenoxy)-phenyl, 4-(3'-protected carboxy phenoxy)-phenyl, 4-(4'-carboxy phenyl amino)-phenyl, 4-(4'-protected carboxy phenyl amino)-phenyl, or 4-(3'-carboxy phenyl amino)-phenyl, 4-(3'-protected carboxy phenyl amino)-phenyl. Also, the term "substituted phenyl" represents disubstituted phenyl groups wherein the substituents are different, for example, 3-methyl-4-hydroxyphenyl, 3-chloro-4-hydroxyphenyl, 2-methoxy-4-bromophenyl, 4-ethyl-2-hydroxyphenyl, 3-hydroxy-4-nitrophenyl, 2-hydroxy 4-chlorophenyl and the like.

[052] The term "C_7 to C_{12} phenylalkyl" denotes a C_1 to C_6 alkyl group substituted at any position by a phenyl, substituted phenyl, heteroaryl or substituted heteroaryl. Examples of such a group include benzyl, 2-phenylethyl, 3-phenyl (n-propyl), 4-phenylhexyl, 3-phenyl (n-amyl), 3-phenyl (sec-butyl) and the like. Preferred C_7 to C_{12} phenylalkyl groups are the benzyl and the phenylethyl groups.

[053] The term "C_7 to C_{12} substituted phenylalkyl" denotes a C_7 to C_{12} phenylalkyl group substituted on the C_1 to C_6 alkyl portion with one or more, and preferably one or two, groups chosen from halogen, hydroxy, protected hydroxy, oxo, protected oxo, amino, protected amino, (monosubstituted) amino, protected (monosubstituted) amino, (disubstituted) amino, guanidino, protected guanidino, heterocyclic ring, substituted heterocyclic ring, C_1 to C_8 alkyl, C_1 to C_8 substituted alkyl, C_1 to C_8 alkoxy, C_1 to C_8 substituted alkoxy, C_1 to C_8 acyl, C_1 to C_8 substituted acyl, C_1 to C_8 acyloxy, nitro, carboxy, protected carboxy, carbamoyl, carboxamide, protected carboxamide, N-(C_1 to C_6 alkyl)carboxamide, protected N-(C_1 to C_6 alkyl)carboxamide, N, N-(C_1 to C_6 dialkyl)carboxamide, cyano, N-(C_1 to C_6 alkylsulfonyl)amino, thiol, C_1 to C_4 alkylthio, C_1 to C_4 alkylsulfonyl groups; and/or the phenyl group may be substituted with one or more, and preferably one or two, substituents chosen from halogen, hydroxy, protected hydroxy, cyano, nitro, C_1 to C_8 alkyl, C_1 to C_8 substituted alkyl, C_1 to C_8 alkoxy, C_1 to C_8 substituted alkoxy, C_1 to C_8 acyl, C_1 to C_8 substituted acyl, C_1 to C_8 acyloxy, carboxy, protected carboxy, carboxymethyl, protected carboxymethyl, hydroxymethyl, protected hydroxymethyl, amino, protected amino, monosubstituted amino, protected monosubstituted amino, disubstituted amino, carboxamide, protected carboxamide, N-(C_1 to C_6 alkyl) carboxamide, protected N-(C_1 to C_6 alkyl) carboxamide, N, N-di(C_1 to C_6 alkyl)carboxamide, trifluoromethyl, N-((C_1 to C_6 alkyl)sulfonyl)amino, N-(phenylsulfonyl)amino, cyclic C_2 to C_8 alkylene or a phenyl group, substituted or unsubstituted, for a resulting biphenyl group. The substituted alkyl or phenyl groups may be substituted with one or more, and preferably one or two, substituents which can be the same or different.

[054] Examples of the term "C_7 to C_{12} substituted phenylalkyl" include groups such as 2-hydroxyphenylmethyl, 3-hydroxyphenylmethyl, 2-methoxyphenylmethyl, 3-methoxyphenyl-methyl, 2,6-difluorophenylmethyl, 2,3-difluorophenylmethyl, 2,6-dichlorophenylmethyl, 2,3-dichlorophenylmethyl, 3,5-dichlorophenylmethyl, 2-hydroxyphenylethyl, 3-hydroxyphenylethyl, 2-methoxyphenylethyl, 3-methoxyphenylethyl, 2,6-difluorophenylethyl, 2,3-difluorophenylethyl,

2,6-dichlorophenylethyl, 2,3-dichlorophenylethyl, 3,5-dichlorophenylmethyl 2-phenyl-1-chloroethyl, 2-(4-methoxyphenyl)ethyl, 4-(2,6-dihydroxy phenyl)n-hexyl, 2-(5-cyano-3-methoxyphenyl)n-pentyl, 3-(2,6-dimethylphenyl)n-propyl, 4-chloro-3-aminobenzyl, 6-(4-methoxyphenyl)-3-carboxy(n-hexyl), 5-(4-aminomethylphenyl)- 3-(aminomethyl)n-pentyl, 5-phenyl-3-oxo-n-pent-1-yl and the like.

[055] The term "heterocycle" or "heterocyclic ring" denotes optionally substituted five-membered to eight-membered rings that have 1 to 4 heteroatoms, such as oxygen, sulfur and/or nitrogen, in particular nitrogen, either alone or in conjunction with sulfur or oxygen ring atoms. These five-membered to eight-membered rings may be saturated, fully unsaturated or partially unsaturated, with fully saturated rings being preferred. Preferred heterocyclic rings include morpholino, piperidinyl, piperazinyl, 2-amino-imidazoyl, tetrahydrofurano, pyrrolo, tetrahydrothiophen-yl, hexamethyleneimino and heptamethyleneimino.

[056] The term "substituted heterocycle" or "substituted heterocyclic ring" means the above-described heterocyclic ring is substituted with, for example, one or more, and preferably one or two, substituents which are the same or different which substituents can be halogen, hydroxy, protected hydroxy, cyano, nitro, C_1 to C_8 alkyl, C_1 to C_8 alkoxy, C_1 to C_8 substituted alkoxy, C_1 to C_8 acyl, C_1 to C_8 acyloxy, carboxy, protected carboxy, carboxymethyl, protected carboxymethyl, hydroxymethyl, protected hydroxymethyl, amino, protected amino, monosubstituted amino, protected monosubstituted amino, disubstituted amino carboxamide, protected carboxamide, N-(C_1 to C_{12} alkyl)carboxamide, protected N-(C_1 to C_6 alkyl)carboxamide, N, N-di(C_1 to C_6 alkyl)carboxamide, trifluoromethyl, N-((C_1 to C_6 alkyl)sulfonyl)amino, N-(phenylsulfonyl)amino, heterocycle or substituted heterocycle groups.

[057] The term "heteroaryl" means a heterocyclic aromatic derivative which is a five-membered or six-membered ring system having from 1 to 4 heteroatoms, such as oxygen, sulfur and/or nitrogen, in particular nitrogen, either alone or in conjunction with sulfur or oxygen ring atoms. Examples of heteroaryls include pyridinyl, pyrimidinyl, and pyrazinyl, pyridazinyl, pyrrolo, furano, thiopheno, oxazolo, isoxazolo, phthalimido, thiazolo and the like.

[058] The term "substituted heteroaryl" means the above-described heteroaryl is substituted with, for example, one or more, and preferably one or two, substituents which are

the same or different which substituents can be halogen, hydroxy, protected hydroxy, cyano, nitro, C_1 to C_8 alkyl, C_1 to C_8 alkoxy, C_1 to C_8 substituted alkoxy, C_1 to C_8 acyl, C_1 to C_8 substituted acyl, C_1 to C_8 acyloxy, carboxy, protected carboxy, carboxymethyl, protected carboxymethyl, hydroxymethyl, protected hydroxymethyl, amino, protected amino, monosubstituted amino, protected monosubstituted amino, disubstituted amino, carboxamide, protected carboxamide, N-(C_1 to C_6 alkyl)carboxamide, protected N-(C_1 to C_6 alkyl)carboxamide, N, N-di(C_1 to C_6 alkyl)carboxamide, trifluoromethyl, N-((C_1 to C_6 alkyl)sulfonyl)amino or N-(phenylsulfonyl)amino groups.

[059] The term "substituted naphthyl" specifies a naphthyl group substituted with one or more, and preferably one or two, moieties either on the same ring or on different rings chosen from the groups consisting of halogen, hydroxy, protected hydroxy, cyano, nitro, C_1 to C_8 alkyl, C_1 to C_6 alkoxy, C_1 to C_8 acyl, C_1 to C_8 acyloxy, carboxy, protected carboxy, carboxymethyl, protected carboxymethyl, hydroxymethyl, protected hydroxymethyl, amino, protected amino, monosubstituted amino, protected monosubstituted amino, disubstituted amino, carboxamide, protected carboxamide, N-(C_1 to C_6 alkyl)carboxamide, protected N-(C_1 to C_6 alkyl)carboxamide, N, N-di(C_1 to C_6 alkyl)carboxamide, trifluoromethyl, N-((C_1 to C_6 alkyl)sulfonyl)amino or N-(phenylsulfonyl)amino.

[060] Examples of the term "substituted naphthyl" includes a mono or di (halo) naphthyl group such as 1, 2, 3, 4, 5, 6, 7 or 8-chloronaphthyl, 2, 6-dichloronaphthyl, 2, 5-dichloronaphthyl, 3, 4-dichloronaphthyl, 1, 2, 3, 4, 5, 6, 7 or 8-bromonaphthyl, 3, 4-dibromonaphthyl, 3-chloro-4-fluoronaphthyl, 1, 2, 3, 4, 5, 6, 7 or 8-fluoronaphthyl and the like; a mono or di (hydroxy) naphthyl group such as 1, 2, 3, 4, 5, 6, 7 or 8-hydroxynaphthyl, 2, 4-dihydroxynaphthyl, the protected-hydroxy derivatives thereof and the like; a nitronaphthyl group such as 3- or 4-nitronaphthyl; a cyanonaphthyl group, for example, 1, 2, 3, 4, 5, 6, 7 or 8-cyanonaphthyl; a mono- or di(alkyl)naphthyl group such as 2, 3, 4, 5, 6, 7 or 8-methylnaphthyl, I, 2, 4-dimethylnaphthyl, I, 2, 3, 4, 5, 6, 7 or 8-(isopropyl)naphthyl, I, 2, 3, 4, 5, 6, 7 or 8-ethylnaphthyl, I, 2, 3, 4, 5, 6, 7 or 8-(n-propyl)naphthyl and the like; a mono or di(alkoxy)naphthyl group, for example, 2, 6-dimethoxynaphthyl, 1, 2, 3, 4, 5, 6, 7 or 8-methoxynaphthyl, 1, 2, 3, 4, 5, 6, 7 or 8-ethoxynaphthyl, I, 2, 3, 4, 5, 6, 7 or 8-(isopropoxy)naphthyl, 1, 2, 3, 4, 5, 6, 7 or 8-(t-butoxy)naphthyl, 3-ethoxy-4-methoxynaphthyl and the like; 1, 2, 3, 4, 5, 6, 7 or 8-trifluoromethylnaphthyl; a mono- or dicarboxynaphthyl or

(protected carboxy)naphthyl group such as 1, 2, 3, 4, 5, 6, 7 or 8-carboxynaphthyl or 2, 4-di(-protected carboxy)naphthyl; a mono-or di(hydroxymethyl)naphthyl or (protected hydroxymethyl)naphthyl such as 1, 2, 3, 4, 5, 6, 7 or 8-(protected hydroxymethyl)naphthyl or 3, 4-di(hydroxymethyl)naphthyl; a mono- or di(amino)naphthyl or (protected amino)naphthyl such as 1, 2, 3, 4, 5, 6, 7 or 8-(amino)naphthyl or 2, 4-(protected amino)-naphthyl, a mono- or di(aminomethyl)naphthyl or (protected aminomethyl)naphthyl such as 2, 3, or 4-(aminomethyl)naphthyl or 2, 4-(protected aminomethyl)-naphthyl; or a mono- or di-(N-methylsulfonylamino) naphthyl such as 1, 2, 3, 4, 5, 6, 7 or 8-(N-methylsulfonylamino)naphthyl. Also, the term "substituted naphthyl" represents disubstituted naphthyl groups wherein the substituents are different, for example, 3-methyl-4-hydroxynaphth-1-yl, 3-chloro-4-hydroxynaphth-2-yl, 2-methoxy-4-bromonaphth-1-yl, 4-ethyl-2-hydroxynaphth-1-yl, 3-hydroxy-4-nitronaphth-2-yl, 2-hydroxy-4-chloronaphth-1-yl, 2-methoxy-7-bromonaphth-1-yl, 4-ethyl-5-hydroxynaphth-2-yl, 3-hydroxy-8-nitronaphth-2-yl, 2-hydroxy-5-chloronaphth-1-yl and the like.

[061] As outlined above R_3 and R_4 may be taken together with nitrogen to form a heterocycle or substituted heterocycle of the following kind aziridine, azetidine, pyrrolidine, 3-methylpyrrolidine, 3-aminopyrrolidine, 3-hydroxypyrrolidine, pyrazolidine, imidazolidine, piperidine, 2-methylpiperidine, 4-carboxypiperidine, 4-(carboxymethyl) piperidine, piperazine, morpholine, azepine, tetrahydroisoquinoline.

[062] The term "C_1 to C_8 acyl" encompasses groups such as formyl, acetyl, propionyl, butyryl, pentanoyl, pivaloyl, hexanoyl, heptanoyl, benzoyl and the like. Preferred acyl groups are acetyl and benzoyl.

[063] The term "C_1 to C_8 substituted acyl" denotes the acyl group substituted by one or more, and preferably one or two, halogen, hydroxy, protected hydroxy, oxo, protected oxo, cyclohexyl, naphthyl, amino, protected amino, monosubstituted amino, protected monosubstituted amino, disubstituted amino, guanidino, heterocyclic ring, substituted heterocyclic ring, imidazolyl, indolyl, pyrrolidinyl, C_1 to C_8 alkoxy, C_1 to C_8 acyl, C_1 to C_8 acyloxy, nitro, C_1 to C_8 alkyl ester, carboxy, protected carboxy, carbamoyl, carboxamide, protected carboxamide, N-(C_1 to C_6 alkyl)carboxamide, protected N-(C_1 to C_6 alkyl)carboxamide, N,N-di(C_1 to C_6 alkyl)carboxamide, cyano, methylsulfonylamino, thiol, C_1 to C_4 alkylthio or C_1 to C_4

alkylsulfonyl groups. The substituted acyl groups may be substituted once or more, and preferably once or twice, with the same or with different substituents.

[064] Examples of C_1 to C_8 substituted acyl groups include 4-phenylbutyroyl, 3-phenylbutyroyl, 3-phenylpropanoyl, 2- cyclohexanylacetyl, cyclohexanecarbonyl, 2-furanoyl and 3-dimethylaminobenzoyl and the like.

[065] The term "C_1 to C_8 alkoxy" as used herein denotes groups such as methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, t-butoxy and like groups. A preferred alkoxy is methoxy. The term "C_1 to C_8 substituted alkoxy" means the alkyl portion of the alkoxy can be substituted in the same manner as in relation to C_1 to C_8 substituted alkyl.

[066] The term "C_1 to C_8 substituted aminoacyl" denotes the acyl group substituted by one or more, and preferably one or two, halogen, hydroxy, protected hydroxy, oxo, protected oxo, cyclohexyl, naphthyl, amino, protected amino, monosubstituted amino, protected monosubstituted amino, disubstituted amino, guanidino, heterocyclic ring, substituted heterocyclic ring, imidazolyl, indolyl, pyrrolidinyl, C_1 to C_8 alkoxy, C_1 to C_8 acyl, C_1 to C_8 acyloxy, nitro, C_1 to C_8 alkyl ester, carboxy, protected carboxy, carbamoyl, carboxamide, protected carboxamide, N-(C_1 to C_6 alkyl)carboxamide, protected N-(C_1 to C_6 alkyl)carboxamide, N,N-di(C_1 to C_6 alkyl)carboxamide, cyano, methylsulfonylamino, thiol, C_1 to C_6 alkylthio or C_1 to C_6 alkylsulfonyl groups. The substituted acyl groups may be substituted once or more, and preferably once or twice, with the same or with different substituents.

[067] This invention provides a pharmaceutical composition comprising an effective amount of at least one compound according to the invention. Such compositions can be administered by various routes, for example oral, subcutaneous, *via* suppositories intramuscular, intravenous or intracerebral. The preferred route of administration would be oral at daily doses of the compound for adult human treatment of about 0.01 -5000 mg, preferably about 1-1500 mg per day. The appropriate dose may be administered in a single dose or as divided doses presented at appropriate intervals for example as two, three four or more subdoses per day.

[068] For preparing pharmaceutical compositions containing at least one compound of the invention, inert, pharmaceutically acceptable carriers are used. The pharmaceutical carrier can be either solid or liquid. Solid form preparations include, for example, powders, tablets, dispersible granules, capsules, cachets, and suppositories.

[069] A solid carrier can be one or more substances which can also act as diluents, flavoring agents, solubilizers, lubricants, suspending agents, binders, or tablet disintegrating agents; it can also be an encapsulating material.

[070] In powders, the carrier is generally a finely divided solid that is in a mixture with the finely divided active component. In tablets, the active compound is mixed with the carrier having the necessary binding properties in suitable proportions and compacted in the shape and size desired.

[071] For preparing pharmaceutical compositions in the form of suppositories, a low-melting wax such as a mixture of fatty acid glycerides and cocoa butter is first melted and the active ingredient is dispersed therein by, for example, stirring. The molten homogeneous mixture is then poured into convenient-sized molds and allowed to cool and solidify.

[[072] Powders and tablets preferably contain between about 5% to about 70% by weight of the active ingredient. Suitable carriers include, for example, magnesium carbonate, magnesium stearate, talc, lactose, sugar, pectin, dextrin, starch, tragacanth, methyl cellulose, sodium carboxymethyl cellulose, a low-melting wax, cocoa butter and the like.

[073] The pharmaceutical compositions can include the formulation of the active compound(s) with encapsulating material as a carrier providing a capsule in which the active component(s) (with or without other carriers) is surrounded by a carrier, which is thus in association with it. In a similar manner, cachets are also included. Tablets, powders, cachets, and capsules can be used as solid dosage forms suitable for oral administration.

[074] Liquid pharmaceutical compositions include, for example, solutions suitable for oral or parenteral administration, or suspensions, and emulsions suitable for oral administration. Sterile water solutions of the active component or sterile solutions of the active component in

solvents comprising water, ethanol, or propylene glycol are examples of liquid compositions suitable for parenteral administration.

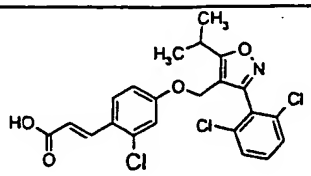
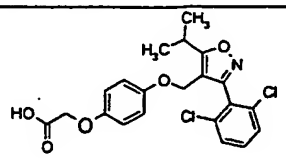
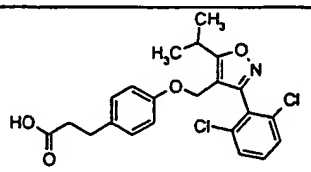
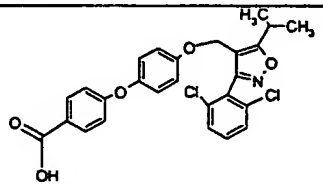
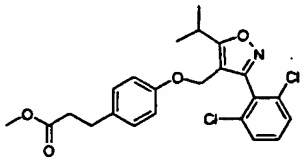
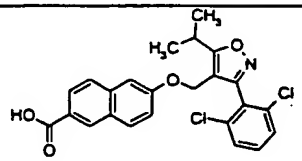
[075] Sterile solutions can be prepared by dissolving the active component in the desired solvent system, and then passing the resulting solution through a membrane filter to sterilize it or, alternatively, by dissolving the sterile compound in a previously sterilized solvent under sterile conditions.

[076] In particular the invention relates to compounds as described above wherein the compounds are capable of binding the NR1H4 receptor protein or a portion thereof as shown in SEQ ID NO. 1 (Fig. 2A) or a mammalian homologue thereof. The compounds can bind to the NR1H4 receptor protein or a portion thereof in a mixture comprising about 10-200 ng of NR1H4 receptor protein or a portion thereof, preferably the ligand binding domain, 20 mM Tris /HCl at pH 7.9; 60 mM KCl; 5 mM $MgCl_2$; 160 ng/µl BSA in a total volume of preferably about 25 µl.

[077] A mammalian receptor protein homologue of the protein according to SEQ ID NO. 1 as used herein is a protein that performs substantially the same function as NR1H4 does in humans and shares at least about 40% sequence identity at the amino acid level, preferably about 50 % sequence identity at the amino acid level more preferably about 65 % sequence identity at the amino acid level, even more preferably about 75 % sequence identity at the amino acid level and most preferably over about 85 % sequence identity at the amino acid level.

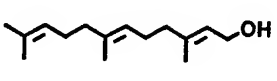
[078] Table 1 shows the structures of preferred compounds according to the invention. The table further shows their respective EC_{50} values (EC50 AVG) as established according to results of multiple experiments, as well as their respective average efficacy (% activity relative to CDCA control agonist).

TABLE 1

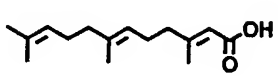
MOLNAME	MOLECULE STRUCTURE	EC50 AVG	EFFIC AVG
LN0000006772		0.05	100
LN0000006767		0.23	117
LN0000006765		0.43	115
LN0000006734		0.6	85
LN0000006764		0.72	108
LN0000000169		2.6	134

[079] Table 2 shows various known FXR ligands. It is apparent from their structures that the inventors have identified novel compounds that are structurally not related to these known ligands.

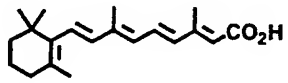
TABLE 2



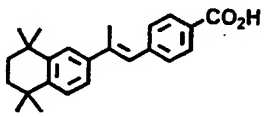
Farnesol



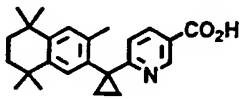
Farnesoic acid



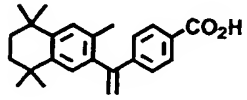
All *trans*-Retinoic acid



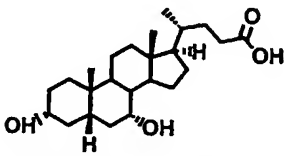
TTNPB



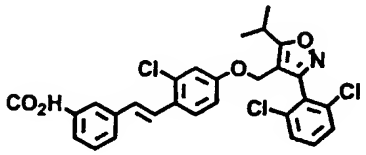
LG 268



LG 69



Carboxysteroids
CDCA



Ethers
GW 4064

[080] As can be seen the compounds of the present invention are structurally unrelated to these known ligands.

[081] The invention in particular concerns a method for prevention or treatment of a NR1H4 receptor protein or NR1H4 receptor protein homologue mediated disease or condition in a mammal comprising administration of a therapeutically effective amount of a compound according to the invention wherein the prevention or treatment is directly or indirectly accomplished through the binding of a compound according to the invention to the NR1H4 receptor protein or to the NR1H4 receptor protein homologue.

[082] The term mediated herein means that the physiological pathway in which the NR1H4 receptor protein acts is either directly or indirectly involved in the disease or condition to be treated or prevented. In the case where it is indirectly involved it could be that, e.g. modulating the activity of NR1H4 by a compound according to the invention influences a parameter which has a beneficial effect on a disease or a condition. One such example is that modulation of NR1H4 activity leads to decreased levels of serum cholesterol or certain lipoproteins which in turn have a beneficial effect on the prevention and treatment of artherosclerosis. Herein a condition is a physiological or phenotypic state which is desirably altered. Another example would be the treatment of cholestatic conditions in which bile flow from the liver to the gut is impaired which results in a tailback of toxic metabolites to the liver. Cholestasis can be a primary condition where bile flow is directly impaired or a secondary condition where a primary impairment in liver function such as liver cirrhosis results in a secondary cholestasis. Agonists that activate NR1H4 resulting in increased bile acid export from the hpeatocyte into the liver canaliculi and subsequent increased bile flow might be used for the treatment of these different types of cholestasis.

[083] In a preferred embodiment of the invention the method for prevention or treatment of a NR1H4 receptor protein mediated disease or condition is applied to a human. This may be male or female.

[084] Listed below are various genes that have been found to be regulated in mammalians by binding of an FXR agonist to the FXR receptor.

[085] Genes down-regulated in liver:
Apolipoprotein A1, ApoA1 (NM000039), plasma proteinase inhibitor alpha-1-inhibitor III group 3(m22360), L-glucono-gamma-lactone oxidase (d12754), Peroxisomal enoyl-CoA:hydrotase-3-hydroxyacyl-CoA bifunctional enzyme (k03249) liver fatty acid binding protein (L-FABP, m13501), CYP4A2(m57719,CYP3A23 (x96721), CYP3A1 (x64401);(b), Cholesterol-7-alpha-hydroxylase, CYP7A1 (RefSeq NM000780, XM 005022, XM 044651, XM 044652), Sodium-taurocholate cotransport protein, ntcp (RefSeq NM003049, XM007466), CYP8B1 (NM004391)

[086] Genes up-regulated in liver:

Small heterodimer partner homolog (d86580), Bile salt export pump, bsep (RefSeq NM 003742, XM 003644, XM 033122), Phospholipid transfer protein, PLTP (RefSeq NM 006227, XM 009490, XM 029929, XM 029930), Carnithine palmitoyltransferase II, CPTII (RefSeq NM 000098, XM 001758, XM 038866, XM 038867), Phenylethanolamine-N-methyltransferase, PNMT (RefSeq NM 002686, XM 008597, XM 049837), insulin-induced growth-response protein CL-6 (I13619), elongation factor 2, EF-2 (y07504), mouse cornichon, protein kinase C receptor (u03390), mitochondrial cytochrome c oxidase (m27315), cystathione gamma-lyase (x53460, d17370), cytosolic phosphoenolypyruvate carboxykinase (k03243), histidase (m58308) S-adenosylmethionine synthetase (x60822), lanosterol 14-alpha-demethylase (u17697), G protein-coupled purinoceptor P2U (146865), hepatic squalene synthetase (m95591), ATP-binding cassette transporter, ABCC2 (Q92887), Apolipoprotein CII, APOCII (NM000483), Dehydroepiandrosterone sulfotransferase (XM049895)

[087] Genes regulated in the intestine:
lipase (x61925),pancreatic lipase (d88534), colipase (m58370), pancreatic phospholipase A-2 (d00036), pancreatic amylase (m24962), carboxypeptidase A1 (m23986), carboxypeptidase A2 (m23721), carboxypeptidase B (m23959), pancreatic trypsin I (j00778), pancreatic cationic trypsinogen (m16624), pancreatic trypsinogen II (v01274), elastase I (v01234, I00112), elastase II (I00118, I00124), I-BABP (I22788), intestinal fatty acid binding protein (FABP, k01180), hepatic squalenesynthetase (m95591), protein kinase C receptor (u003390), elongation factor 2, EF-2 (y07504), Small heterodimer partner homolog (d86580)

[088] Pharmaceutical compositions generally are administered in an amount effective for treatment or prophylaxis of a specific condition or conditions. Initial dosing in human is accompanied by clinical monitoring of symptoms, such symptoms for the selected condition. In general, the compositions are administered in an amount of active agent of at least about 100 µg/kg body weight. In most cases they will be administered in one or more doses in an amount not in excess of about 20 mg/kg body weight per day. Preferably, in most cases, doses is from about 100 µg/kg to about 5 mg/kg body weight, daily.

[089] For administration particularly to mammals, and particularly humans, it is expected that the daily dosage level of active agent will be 0.1 mg/kg to 10 mg/kg and typically around 1 mg/kg.

[090] By "therapeutically effective amount" is meant a symptom- alleviating or symptom -reducing amount, a cholesterol-reducing amount, an amount that overcomes cholestatic conditions, a protein and/or carbohydrate digestion-blocking amount and/or a de novo cholesterol biosynthesis-blocking amount of a compound according to the invention.

[091] FXR is proposed to be a bile acid sensor. As a result, it modulates both, the synthetic output of bile acids from the liver and their recycling in the intestine, by regulating bile acid binding proteins. In one embodiment of the invention the invention concerns a method for regulating the bile transport system in a mammal, in a preferred embodiment a human, which comprises activating the NR1H4 receptor with a therapeutically effective amount of a compound according to the invention.

[092] Likewise the invention concerns a method of treating in mammal a disease which is affected by cholesterol, triglyceride, bile acid levels or bile flow comprising administering to a mammal in need of such treatment a therapeutically effective amount of a compound according to the invention.

[093] Accordingly, the compounds according to the invention may also be used as a method of prevention or treatment of mammalian atherosclerosis, gallstone disease (cholelithiasis), primary and secondary forms of cholestasis, lipid disorders, obesity or cardiovascular disorders such as coronary heart disease or stroke.

[094] The invention further concerns a method of blocking in a mammal the cholesterol absorption in the intestine of a mammal in need of such blocking comprising administering to a mammal in need of such treatment a therapeutically effective amount of a compound according to the invention. The invention may also be used to treat obesity in humans.

[095] The Farnesoid X Receptor alpha is a prototypical type 2 nuclear receptor which activates genes upon binding to the promoter region of target genes in a heterodimeric fashion with Retinoid X Receptor. The relevant physiological ligands of NR1H4 are bile acids. The present compounds according to the invention have been demonstrated to have a high binding efficacy as measured as IC50 in the range 400 nM to 1000 nM as well as agonistic and / or

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antagonistic properties. Consequently they may be applied to regulate genes that participate in bile acid homeostasis as well as other downstream regulated genes. Examples of physiological functions in which such genes are involved are but are not limited to lipid absorption, cholesterol biosynthesis, cholesterol transport or binding, bile acid synthesis, bile acid transport or binding, proteolysis, amino acid metabolism, glucose biosynthesis, protein translation, electron transport, and hepatic fatty acid metabolism. FXR often functions in vivo as a heterodimer with the Retinoid X Receptor. Published FXR agonists such as the Glaxo SmithKline compound "GW 4064" and published FXR antagonists such as guggulsterone [4,17(20)-pregnadiene-3,16-dione] are known to influence the regulation of various liver genes. Genes found to be regulated by GW 4064 can be found in figure 6. Thus, the invention also concerns a method of modulating a gene whose expression is regulated by the NR1H4 receptor in a mammal comprising administration of a therapeutically effective amount of a compound according to the invention to said mammal.

[096] It is known that the orphan receptor FXR can bind the response element of the shp gene as a heterodimer with RXR (9-cis retinoic acid receptor) and the SHP-protein, in turn, prevents efficient transcription from the cyp7a1 promoter (Lu et al., Mol Cell, 6(3):505-17; Goodwin et al. Mol Cell, 6(3), 717-26, 2000). . Another gene that is repressed via SHP upon FXR activation is the Sodium / Bile Acid Cotransporter gene ntcp, a membrane transport protein which is required for the import of conjugated bile acids into the hepatocyte (Denson et al., Gastroenterology;121(1):218-20, 2001). The gene for the Bile Salt Export Pump, a membrane transporter responsible for the secretion of bile acids into the gall is directly activated by FXR (Ananthanarayanan et al., J Biol Chem, 3;276(31):28857-28865, 2001). Consequently, the invention likewise concerns a method for lowering the expression of cholesterol 7-alpha-hydroxylase and NTCP and increasing expression of BSEP and/ or MDR2 (=multidrug resistance protein 2) in parallel by use of the compounds according to the invention. This is believed to be the ideal profile of an anti-cholestatic compound (Kullack-Ublick, et al., J Hepatol (2000) 32 Suppl 1:3-18). In one embodiment the invention concerns a method for enhancing the expression of the Intestinal Bile Acid Binding Protein (I-BABP) (Grober et al., J Biol Chem, 15;274(42):29749-54, (1999) and/or the activity of the canicular bile salt excretion pump.

[097] The compounds according to the invention may be used as medicaments, in particular for the manufacture of a medicament for the prevention or treatment of a NR1H4

receptor protein or NR1H4 receptor protein homologue mediated disease or condition in a mammal wherein the prevention or treatment is directly or indirectly accomplished through the binding of the compound according to the invention to the NR1H4 receptor protein or NR1H4 receptor protein homologue. These pharmaceutical compositions contain 0,1 % to 99,5 % of the compound according to the invention, more particularly 0,5 % to 90 % of the compound according to the invention in combination with a pharmaceutically acceptable carrier.

[098] The invention concerns also the use of a compound according to the invention for the manufacture of a medicament for the prevention or treatment of a NR1H4 receptor protein mediated disease or condition wherein the mammal described above is a human. The medicament may be used for regulating the bile transport system in a mammal preferentially a human by activating the NR1H4 receptor, for regulating levels of cholesterol, triglyceride, bile acids and bile flow in mammals, preferentially humans. The medicament may be used for the treatment of atherosclerosis, gallstone disease (cholelithiasis), cholestasis, lipid disorders, obesity or a cardiovascular disorder.

[099] The further concerns the use of a compound according to the invention for the manufacture of a medicament capable for blocking in a mammal, preferentially a human the cholesterol absorption in the intestine. Further the claimed compound may be used for the manufacture of a medicament for treating obesity in humans and for modulating a gene whose expression is regulated by the NR1H4 receptor (see details above and figures). The invention further concerns the use of a compound according to the invention for the manufacture of anticancer medicaments. The anticancer effects of such medicaments could be excerted by selective inhibition of cell proliferation and induction of apoptosis of tumor cells in a way similar to described activities for certain bisphosphonates (Alberts DS, et al., Clin Cancer Res 2001 May;7(5):1246-50)

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EXAMPLE 1: *in vitro* screening for compounds which influence FXR binding to coactivators.

[100] For screening purposes a fragment of the open reading frame of human FXR alpha (NR1H4 - (Acc. No:AF384555)) encoding aminoacids 187-472 was amplified by standard RT PCR procedures (see figures; SEQ ID NO. 1 and 2). Starting material was total RNA derived from human liver. The resulting cDNA obtained after reverse transcription was subsequently cloned using the Gateway™ recombination technology (Invitrogen, USA) into the expression plasmid pDest15 (Invitrogen, USA). This construct was used to express a recombinant GST-FXR fusion protein in E.coli (BL21 strain). A pDEST 17 derivative clone harboring an additional sequence encoding amino acids 548-878 of human TIF2 (Acc. No: XM_011633 RefSeq) was constructed using Gateway™ recombination technology (Invitrogen, USA) in order to obtain a construct which was used to express recombinant His-tagged TIF2 fragment could be expressed in E. coli. For E. coli expression of both constructs, plasmid DNA was transformed into chemically competent E. coli BL21 (Invitrogen, USA) and cells were grown to an OD600 of 0.4-0.7 before expression was induced by addition of 0,5 mM IPTG according instructions of the manufacturer (Invitrogen). After induction for 8 hours at 30°C cells were harvested by centrifugation for 10 minutes at 5000 x g. Fusion proteins were affinity purified using Glutathion sepharose (Pharmacia) or Ni-NTA Agarose (QIAGEN) according to the instructions of the respective manufacturer. Recombinant proteins were dialyzed against 20 mM Tris/HCL pH 7.9; 60 mM KCl; 5 mM $MgCl_2$; 1 mM DTT, 0,2 mM PMSF; 10% glycerol. The TIF2 fragment was subsequently biotinylated by addition of 40-120 µl of a Biotinamidocaproate N-Hydroxysuccinimide-ester (Sigma) solution (20 mg/ml in DMSO). Overhead rotating samples were incubated for 2 hours at room temperature. Unincorporated label was then separated using G25 Gel filtration chromatography (Pharmacia Biotech, Sweden). Protein containing fractions from the column were pooled and tested for activity in the assay as described below.

[101] For screening of compound libraries as provided for by the methods shown below in the examples for substances which influence the FXR/Tif 2 interaction, the Perkin Elmer LANCE technology was applied. This method relies on the binding dependent energy transfer from a donor to an acceptor fluorophore attached to the binding partners of interest. For ease of handling and reduction of background from compound fluorescence LANCE technology

makes use of generic fluorophore labels and time resoved detection (for detailed description see Hemmilä I, Blomberg K and Hurskainen P, Time-resolved resonance energy transfer (TR-FRET) principle in LANCE, Abstract of Papers Presented at the 3 rd Annual Conference of the Society for Biomolecular Screening, Sep., California (1997).

[102] For screening, 20-200 ng of biotinylated Tif 2 fragment and 10-200 ng of GST-FXR fragment were combined with 0.5-2 nM LANCE Eu-(W1024) labelled anti-GST antibody (Perkin Elmer) and 0,5-2µg of Highly fluorescent APC-labelled streptavidin (Perkin Elmer) in the presence of 50µM of individual compounds to be screened in a total volume of 25 µl of 20 mM Tris /HCl pH 7.9; 60 mM KCl; 5 mM MgCl2; 160ng/µl BSA. DMSO content of the samples was kept below 4%. Samples were incubated for a minimum of 60 minutes in the dark at room temperature in FIA-Plates black 384well med. binding (Greiner).

[103] The LANCE signal was detected by a Perkin Elmer VICTOR2V™ Multilabel Counter applying the detection parameters listed in Fig. 2. The results were visualized by plotting the ratio between the emitted light at 665 nm and at 615 nm. For every batch of recombinant proteins amount of proteins and labeling reagents giving the most sensitive detection of hits was determined individually by analysis of dose response curves for chenodeoxycholic acid.

TABLE 1: Measurement parameters employed by a Wallace VICTOR2V™ Multilabel Counter

Number of repeats 1
plate: GREINER FIA-Plate black 384 well med. binding
Measurement height ..... 3.50 mm
Label technology ..... TR-F Lance
Emission filter name D615
Emission filter slot A1
Emission aperture ..... Normal
Excitation filter D340
Delay ..... 50 µs
Window time ..... 400 µs

Cycle ..... 1000 µs
Light integrator capacitors 1
Light integrator ref. level 95
Flash energy area ..... High
Flash energy level 223
Flash absorbance measurement ..... No
Beam ..... Normal
Label technology ..... TR-F Lance
Emission filter name D665
Emission filter slot A8
Emission aperture ..... Normal
Excitation filter D340
Delay ..... 50 µs
Window time ..... 400 µs
Cycle ..... 1000 µs
Light integrator capacitors 1
Light integrator ref. level 95
Flash energy area ..... High
Flash energy level 223
Flash absorbance measurement ..... No
Beam ..... Normal

EXAMPLE 2: Experimental procedure for the preparation of the compounds according to the invention.

[104] The following steps describe the experimental procedure for the preparation of the compounds according to the invention. The synthesis scheme is shown in Fig. 1.

Step 1: Synthesis of Dicholorobenzaldehyde oxime (compound 3).

[105] A solution of 2,6-dichlorobenzaldehyde (compound 2) (0.14mole) in ethanol (200mL) was added to a solution of hydroxylamine hydrochloride (0.16mole) and sodium hydroxide (0.16mole) in water (100ml). The resulting mixture was stirred at 90°C for 24 hours. The volume of the reaction mixture was reduced in vacuo by ~30 mL, which induced a precipitate. The white solids were collected by filtration and washed with water (2x100 mL) to yield (96%) of dicholorobenzaldehyde oxime (compound 3).

Step 2: Synthesis of 3-(2,6-dichlorophenyl)-4-carbomethoxy-5-isopropyl-isoxazole (compound 5).

[106] N-chlorosuccinimide (0.07mole) was added at room temperature to a solution of 2,6-dichlorobenzaldehyde oxime (compound 3) (0.07mole) in DMF (150mL). The reaction was slightly exothermic and the reaction mixture turned into dark yellow color. The reaction mixture was stirred for an additional one hour, and then the contents were poured into water (200mL) and extracted with diethyl ether (300mL). The organic layer was washed with water (3x100mL) and brine (50mL), dried (Na_2SO_4) and concentrated to obtain 2,6-dicholorophenylhydroximic chloride (94%). A stirred solution of methyl isobutyryl acetate (compound 4) (15.6mmol) in tetrahydrofuran (15mL) was treated with a solution of sodium methoxide (31.5mL, 0.5M in methanol) followed by a solution of 2,6-dicholorophenylhydroximic chloride (15.6 mmol) in tetrahydrofuran (5 mL). After stirring at room temperature for 16 hours the solvent was removed in vacuo. The resulting residue was partitioned with diethyl ether (100 mL) and water (100 mL). The ether layer was washed with brine (50 mL), dried (Na_2SO_4), and concentrated to obtain a

residue which was purified by flash column chromatography on silica gel using 10% ethyl acetate in hexane as mobile phase to yield 3.1g(62%) of 3-(2,6-dichlorophenyl)-4-carbomethoxy-5-isopropyl-isoxazole (compound 5).

Step 3: Reduction.

[107] A solution of 3-(2,6-dichlorophenyl)-4-carbomethoxy-5-isopropyl-isoxazole (compound 5) (27 mmol) in tetrahydrofuran (60 mL) was cooled to 0°C under a nitrogen atmosphere. A solution of diisobutylaluminum hydride (38 ml 2.1 eq, 1.5 M in toluene) was added dropwise. The reaction mixture was allowed to warm up to room temperature and was stirred for an additional 16 hours. The reaction mixture was cooled to 0°C and quenched with methanol (2 mL). When water (20 mL) was added dropwise a gelatinous precipitate was obtained. Sodium hydroxide (30 mL, 2N) was then added and the material was filtered through celite. The filtrate was extracted with ethyl acetate, washed with water and saturated sodium chloride solution, dried (Na_2SO_4), and concentrated to obtain the alcohol (compound 6).

Step 4: Mitsunobu Reaction.

[108] To a solution of phenol (compound 7) (0.6 mmol), isoxazole (compound 6) (0.6mmol) and triphenylphosphine (0.6mmol) in dichloromethane (10mL), diisopropyl azodicarboxylate((0.6mmol) was added dropwise. A brief exotherm was observed and the reaction mixture was stirred at room temperature for 4 hours. The reaction mixture was concentrated to a residue and was purified by flash column chromatography using 20% ethyl acetate in hexane as eluant to obtain 8 in 85% yield.

Step 5: Ester Hydrolysis.

[109] Lithium hydroxide (0.8 mmol, 1M in water) was added to a solution of ester (compound 8) (0.2 mmol) in THF (5 mL). The reaction mixture was stirred vigorously at room temperature for 24 hours. THF was removed *in vacuo* and the reaction mixture was extracted with ethyl acetate, dried (Na_2SO_4), and concentrated to obtain an oil which was purified by flash column chromatography to form compound 8.

[110] All of the final products were analyzed using an Evaporative Light Scattering Detector (ELSD) detection to determine purity. One skilled in the art will be able to arrive at the compounds claimed herein making use of the above protocol.

[111] A compound according to the invention (experiments shown were done with MOLSTRUCTURE LN 6734; see Table 1 for structural formula) can mediate transactivation of FXR-mediated transcription in a HEK293 reporter cell line. Stable HEK293FXR reporter cell lines were generated by stably transfecting with the pTRexDest30 (Invitrogen) derivatives pTRexDest30-hFXR, pTRexDest30-hRXR and the pGL2promoter (Promega) derivative, pGL2promoter-FXRRE. The full length human FXR (accession U68233) and the full length human RXRα (accession P19793) were cloned into the pTRexDest30 applying the manufacturer protocols for the Gateway™ system (Invitrogen).

[112] The FXR response elements were (upper case and underlined) 5' - ccca**GGGTGAaTAACCT**cggggctctgtccctccaatccca**GGGTGAaTAACCT**cggg 3' (SEQ ID NO. 5) was created from the human IBAB-P promoter (Grober et al 1999, JBC 274, pp. 29749-29754) and integrated into the reporter plasmid pGL2promoter (Promega) according to standard methods known to those skilled in the art. A stable clone was selected and seeded at a density of $1x10^4$ cells per well in 96 well plates. Luciferase reporter activity were determined in triplicates from extracts of cells after incubating cells in culture medium (DMEM [Gibco-BRL] + 10% FCS [PAA laboratories]) for 16 hours (5% CO_2, 37°C) containing 0,5% DMSO (control) or 0,5% DMSO with increasing concentrations of LN6691 (Fig. 3).
The EC50 value derived in this experiment is 1.3 µM and the relative efficacy compared to the GW4064 as a control compound is about 110% (Fig 3).

[113] Preferred examples of compounds of the invention are shown below in Table 4, together with their dose response curves done for LN6991 and GW4064, CDCA and LN12996 as control compounds are shown in Fig. 3.

TABLE 4

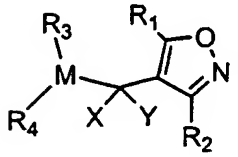
μM	CDCA	LN0000000169	LN0000006734	TR 0800012996	GW 4064
50	6310.0	13375.7	13309.5	5626.0	2159.3
10	3300.7	6829.3	8523.5	8286.0	4030.3
2	1784.0	5775.7	6330.5	4341.0	6084.0
1	1597.7	4600.0	5360.5	3118.0	7440.7
0.4	1421.8	2754.0	2991.5	2491.0	8416.3
0.08	1256.5	1630.0	2153.0	1896.0	6704.7
0.016	1294.3	1333.0	2077.0	2132.0	3319.0
0	1257.7	1396.3	1748.8	1971.1	1274.1

All data are measured in triplicates
max standard deviation +/- 20%

[114] While the salient features of the invention have been illustrated and described with respect to particular embodiments, it should be readily apparent that modifications can be made within the spirit and scope of the invention, and it is, therefore, not desired to limit the invention to the exact details shown and described.

What is claimed is:

1. A compound including or a pharmaceutical acceptable salt or solvate thereof,



(I)

wherein:

R_1 is hydrogen, C_1 to C_8 alkyl, C_1 to C_8 substituted alkyl, phenyl, substituted phenyl, C_5 to C_6 heteroaryl, C_5 to C_6 substituted heteroaryl, naphthyl or substituted naphthyl;

R_2 is phenyl, substituted phenyl, C_5 to C_6 heteroaryl, C_5 to C_6 substituted heteroaryl, heterocycle, substituted heterocycle, naphthyl or substituted naphthyl;

R_3 is absent, or if present, is hydrogen, C_1 to C_8 alkyl, C_1 to C_8 substituted alkyl, C_7 to C_{12} alkylphenyl or C_7 to C_{12} substituted phenylalkyl, biphenyl, substituted biphenyl, biphenyl ether, substituted biphenyl ether, biphenyl amine, substituted biphenyl amine, naphthyl or substituted naphthyl;

R_4 is absent, or if present, is hydrogen, C_1 to C_8 alkyl, C_1 to C_8 substituted alkyl, C_7 to C_{12} alkylphenyl or C_7 to C_{12} substituted phenylalkyl, biphenyl, substituted biphenyl, biphenyl ether, substituted biphenyl ether, biphenyl amine, substituted biphenyl amine, naphthyl or substituted naphthyl;

R_3 and R_4 may be taken together with nitrogen to form a heterocycle or substituted heterocycle;

M is O or N or S; and if M is O or S, one of R_3 or R_4 must be absent; and

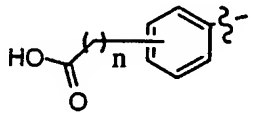
X and Y are both hydrogen or both methyl, or together represent a carbonyl group.

2. The compound of claim 1, wherein:

R_1 is hydrogen, C_1 to C_8 alkyl, phenyl, substituted phenyl, C_5 to C_6 heteroaryl, C_5 to C_6 substituted heteroaryl, naphthyl or substituted naphthyl;

R_2 is substituted phenyl, C_5 to C_6 heteroaryl, or C_5 to C_6 substituted heteroaryl;

R_3 is a formula having the following formula (II),



(II);

R4 is absent;

M is O; and

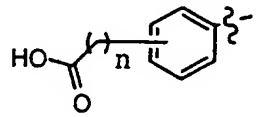
X and Y are both hydrogen.

3. A compound of claim 2, wherein:

R_1 is hydrogen, C_1 to C_8 alkyl, phenyl, substituted phenyl, C_5 to C_6 heteroaryl, C_5 to C_6 substituted heteroaryl, naphthyl or substituted naphthyl;

R_2 is substituted phenyl, C_5 to C_6 heteroaryl, or C_5 to C_6 substituted heteroaryl;

R_3 is a formula having the following formula (II),



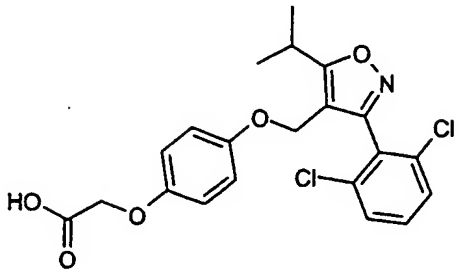
(II);

R4 is hydrogen;

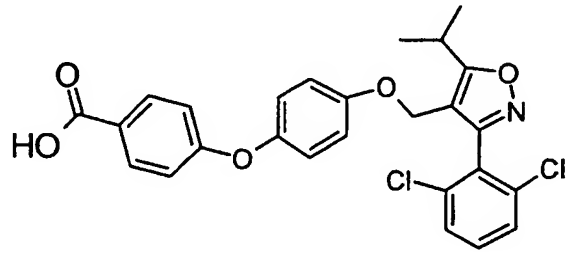
M = N; and

X and Y are both hydrogen.

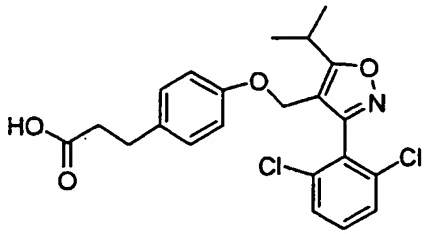
4. The compound according to any of the claims 1 to 3 wherein the compound is



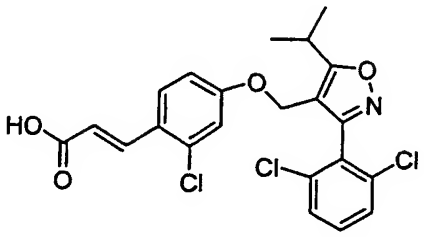
5. The compound according to any of the claims 1 to 3, wherein the compound is



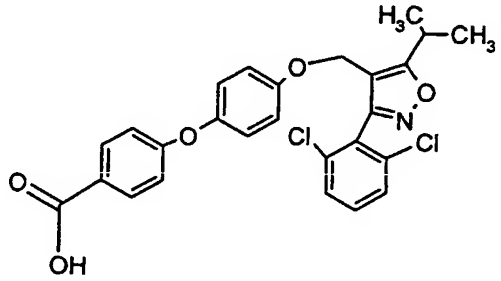
6. The compound according to any of the claims 1 to 3, wherein the compound is



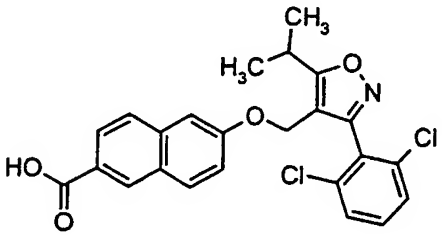
7. The compound according to any of the claims 1 to 3, wherein the compound is



8. The compound according to any of the claims 1 to 3, wherein the compound is



9. The compound according to any of the claims 1 to 3, wherein the compound is



10. A compound according to any of claims 1 to 9 for use as a medicament.

11. A compound according to any of claims 1 to 9 wherein said compound is capable of binding the human NR1H4 receptor protein or a portion thereof or a mammalian homologue thereof according to SEQ ID NO. 1.

12. Use of a compound according to any of claims 1 to 9 for the manufacture of a medicament for the treatment of a NR1H4 receptor mediated or treatable disease or condition in a mammal comprising administration of a therapeutically effective amount of a compound according to any of claims 1 to 9.

13. Use of a compound according to any of claims 1 to 9 for the manufacture of a medicament for regulating bile flow or the bile acid transport system in a mammal by activating or repressing the NR1H4 receptor with a therapeutically effective amount of a compound according to any of claims 1 to 9.

14. Use of a compound according to any of claims 1 to 9 for the manufacture of a medicament for regulating blood levels of cholesterol, lipoproteins, phospholipids, triglycerides, or bile acids and/or bile flow or bile levels of cholesterol, phospholipids or bile acids in a mammal in need of such treatment with a therapeutically effective amount of a compound according to any of claims 1 to 9.

15. Use of a compound according to any of claims 1 to 9 for the manufacture of a medicament for treating cholestatic conditions such as primary biliary cirrhosis (PBC), progressive familiary cholestasis (PFIC), estrogen or drug induced cholestasis, any form of extrahepatic cholestasis, or secondary forms of cholestasis, atherosclerosis, gallstone disease (cholelithiasis), lipid disorders, obesity or a cardiovascular or metabolic disorder in a mammal in need of such treatment with a therapeutically effective amount of a compound according to any of claims 1 to 9.

16. Use of a compound according to any of claims 1 to 9 for the manufacture of a medicament for treating in a mammal malign proliferative diseases such as cancer which can be treated or cured by inducing apoptosis in the affected cells

or tissues comprising administering to a mammal in need of such treatment a therapeutically effective amount of a compound according to any of claims 1 to 9.

17. Use of a compound according to any of claims 1 to 9 for the manufacture of a medicament for treating in a mammal conditions of drug resistance that arise during drug treatment of disorders such as cancer or infectious diseases, or during continous administration of contraceptive drugs comprising administering to a mammal in need of such treatment a therapeutically effective amount of a compound according to any of claims 1 to 9.

18. Use of a compound according to any of claims 1 to 9 for the manufacture of a medicament capable of blocking in a mammal cholesterol absorption or capable of reducing the bile acid reabsorption in the intestine in a mammal in need of such treatment comprising administration of a therapeutically effective amount of a compound according to any of claims 1 to 9.

19. Use of a compound according to any of claims 1 to 9 for the manufacture of a medicament for regulating the expression of NR1H4 responsive genes such as cholesterol-7-alpha hydroxylase (cyp7a1), sterol-12-alpha hydroxylase (cyp8b1), small heterodimer partner (shp), phospholipid transfer protein (pltp), bile salt export pump (bsep), sodium-taurocholate co-transporter (ntcp), organic anion transport proteins 1 and 2 (oatp1 and -2), canalicular multidrug resistance protein 2 (mdr2) or other genes that are members of the cytochrom P450 family or members of the ABC-transporter family or members of the MDR class III multidrug resistance proteins or members of the MRP multidrug resistance protein family or members of the nuclear receptor gene family through activating or repressing the NR1H4 receptor in a mammal in need of such treatment by a therapeutically effective amount of a compound according to any of claims 1 to 9.

20. Use of a compound according to any of claims 1 to 9 for the manufacture of a medicament for modulating the expression of the intestinal bile acid binding protein (IBABP) in intestinal mucosa cells and/or cholangiocytes by the NR1H4 receptor in a mammal in need of such treatment by a therapeutically effective amount of a compound according to any of claims 1 to 9.

21. Use according to any of claims 12 to 20 wherein the mammal is a human.

22. A method for prevention or treatment of a disease or condition which is mediated or can be adressed by the NR1H4 receptor in a mammal comprising administration of a therapeutically effective amount of a compound according to any of claims 1 to 9.

23. A method for regulating bile flow or the bile acid transport system in a mammal which comprises activating or repressing the NR1H4 receptor with a therapeutically effective amount of a compound according to any of claims 1 to 9.

24. A method of treating in a mammal a disease or condition which is affected by impaired blood levels of cholesterol, lipoproteins, phospholipids, triglycerides, or bile acids and/or impaired bile flow or impaired bile levels of cholesterol, phospholipids or bile acids comprising administering to a mammal in need of such treatment a therapeutically effective amount of a compound according to any of claims 1 to 9.

25. A method of treating in a mammal cholestatic conditions such as primary biliary cirrhosis (PBC), progressive familiary cholestasis (PFIC), estrogen or drug induced cholestasis, any form of extrahepatic cholestasis, or secondary forms of cholestasis, atherosclerosis, gallstone disease, lipid disorders, obesity or a

cardiovascular or metabolic disorder comprising administering to a mammal in need of such treatment a therapeutically effective amount of a compound according to any of claims 1 to 9.

26. A method for treating in a mammal malign proliferative diseases such as cancer which can be treated by inducing apoptosis in the affected cells or tissues comprising administering to a mammal in need of such treatment a therapeutically effective amount of a compound according to any of claims 1 to 9

27. A method for treating in a mammal conditions of drug resistance that arise during drug treatment of disorders such as cancer or infectious diseases, or during continous administration of contraceptive drugs comprising administering to a mammal in need of such treatment a therapeutically effective amount of a compound according to any of claims 1 to 9.

28. A method of blocking in a mammal the cholesterol absorption or bile acid re-absorption in the intestine of a mammal in need of such blocking comprising administering to a mammal in need of such treatment a therapeutically effective amount of a compound according to any of claims 1 to 9.

29. A method for regulating the expression of NR1H4 responsive genes such as cholesterol-7-alpha hydroxylase (cyp7a1), sterol-12-alpha hydroxylase (cyp8b1), small heterodimer partner (shp), phospholipid transfer protein (pltp), bile salt export pump (bsep), sodium-taurocholate co-transporter (ntcp), organic anion transport proteins 1 and 2 (oatp1 and -2), canalicular multidrug resistance protein 2 (mdr2) or other genes that are members of the cytochrom P450 family or members of the ABC-transporter family or members of the MDR class III multidrug resistance proteins or members of the MRP multidrug resistance protein family or members of the nuclear receptor gene family by the NR1H4

receptor in a mammal comprising administering a therapeutically effective amount of a compound according to any of claims 1 to 9.

30. A method for modulating the expression of the intestinal bile acid binding protein (IBABP) in intestinal mucosa cells and/or cholangiocytes by the NR1H4 receptor in a mammal comprising administering a therapeutically effective amount of a compound according to any of claims 1 to 9.

31. A method according to any of claims 22 to 30 where the mammal is a human.

FIG. 1

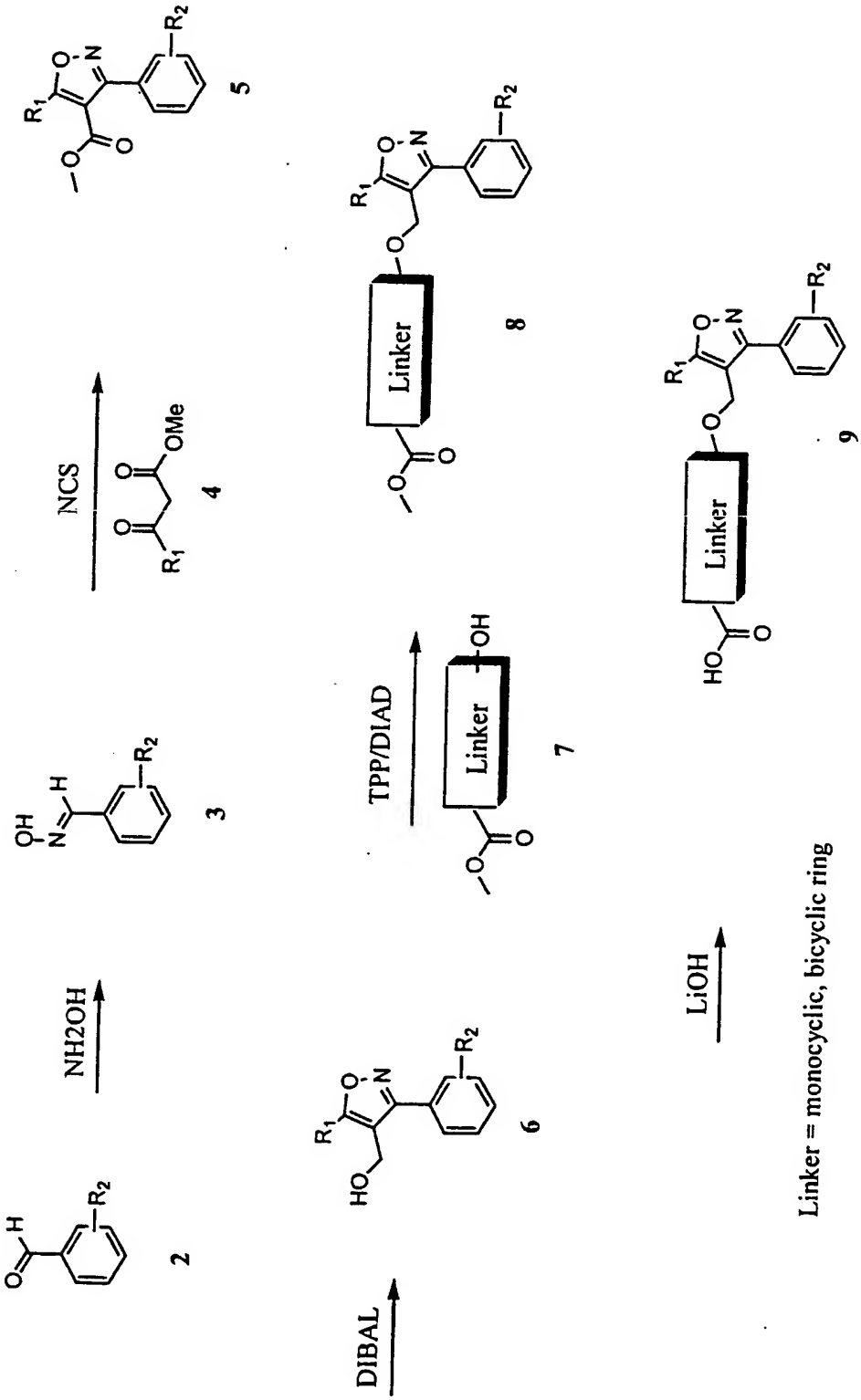


Fig. 1

Linker = monocyclic, bicyclic ring

Fig. 2 A

SEQ ID NO. 1

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ECRLRKCKEM GMLAECMYTG LLTEIQCKSK RLRKNVKQHA DQTVNEDSEG RDLRQVTSTT     240
KSCREKTELT PDQQTLLHFI MDSYNKQRMP QEITNKILKE EFSAEENFLI LTEMATNHVQ     300
VLVEFTKKLP GFQTLDHEDQ IALLKGSAVE AMFLRSAEIF NKKLPSGHSD LLEERIRNSG     360
ISDEYITPMF SFYKSIGELK MTQEEYALLT AIVILSPDRQ YIKDREAVEK LQEPLLDVLQ     420
KLCKIHQPEN PQHFACLLGR LTELRTFNHH HAEMLMSWRV NDHKFTPLLC EIWDVQ         476
```

FIG 2B

SEQ ID NO. 2

```
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ctggaagtgg aaccatactc gcaatacagc aatgttcagt ttccccaagt tcaaccacag      180
atttcctcgt catcctatta ttccaacctg ggtttctacc cccagcagcc tgaagagtgg      240
tactctcctg gaatatatga actcaggcgt atgccagctg agactctcta ccagggagaa      300
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aagtcatgca gggagaaaac tgaactcacc ccagatcaac agactcttct acattttatt      780
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FIG. 2C

SEQ ID NO. 3

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IPPSQFSPAG SLHSPVGVCS STGNSHSYTN SSLNALQALS EGHGVSLGSS LASPDLKMGN     360
LQNSPVNMNP PPLSKMGSLD SKDCFGLYGE PSEGTTGQAE SSCHPGEQKE TNDPNLPPAV     420
SSERADGQSR LHDSKGQTKL LQLLTTKSDQ MEPSPLASSL SDTNKDSTGS LPGSGSTHGT     480
SLKEKHKILH RLLQDSSSPV DLAKLTAEAT GKDLSQESSS TAPGSEVTIK QEPVSPKKKE     540
NALLRYLLDK DDTKDIGLPE ITPKLERLDS KTDPASNTKL IAMKTEKEEM SFEPGDQPGS     600
ELDNLEEILD DLQNSQLPQL FPDTRPGAPA GSVDKQAIIN DLMQLTAENS PVTPVGAQKT     660
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RPSYATLRMQ PRPGLRPTGL VQNQPNQLRL QLQHRLQAQQ NRQPLMNQIS NVSNVNLTLR    1020
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FIG. 2D

SEQ ID NO. 4

```
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FIG. 3

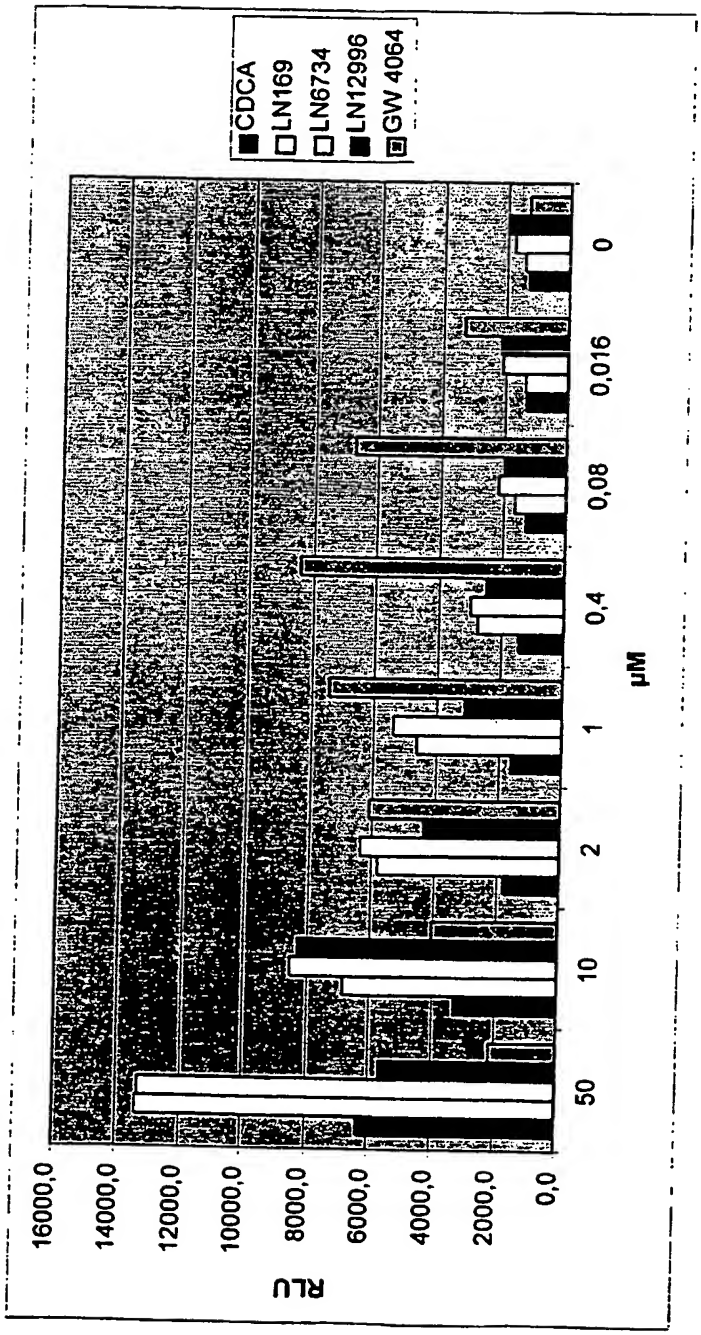

Fig. 4

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2	1784,0	5775,7	6330,5	4341,0	6084,0
1	1597,7	4600,0	5360,5	3118,0	7440,7
0,4	1421,8	2754,0	2991,5	2491,0	8416,3
0,08	1256,5	1630,0	2153,0	1896,0	6704,7
0,016	1294,3	1333,0	2077,0	2132,0	3319,0
0	1257,7	1396,3	1748,8	1971,1	1274,1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/25437

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A61K 31/42; C07D 261/08

US CL :514/378; 548/247, 249

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/378; 548/247, 249

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN CAS ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Chem. Astr., Vol. 121, No. 23, 05 December 1994 (Columbus OH, USA), page 39, column 1, the abstract No. 271343d, LIM, JUNG KI et al. 'Analgesic, anti-inflammatory and antiviral effects of melandrin derivatives.' Yakhak Hoechi. 1994, 38(3), 345-50 (Korean).	1-9

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

18 SEPTEMBER 2002

Date of mailing of the international search report

17 DEC 2002

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
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Form PCT/ISA/210 (second sheet) (July 1998)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/25437

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 10-31
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.